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Emerging understanding of the mechanism of action of Bronchial Thermoplasty in asthma

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ABSTRACT

Bronchial Thermoplasty (BT) is an endoscopic treatment for moderate-to-severe asthma patients who are uncontrolled despite optimal medical therapy. Effectiveness of BT has been demonstrated in several randomized clinical trials. However, the asthma phenotype that benefits most of this treatment is unclear, partly because the mechanism of action is incompletely understood. BT was designed to reduce the amount of airway smooth muscle (ASM), but additional direct and indirect effects on airway pathophysiology are expected. This review will provide an overview of the different components of airway pathophysiology including remodeling, with the ASM as the key player. Current concepts in the understanding of BT clinical effectiveness with a focus on its impact on airway remodeling will be reviewed.

1. Introduction

Severe asthma is defined as uncontrolled asthma despite, or controlled asthma requiring, the use of high dose inhaled corticosteroids (ICS) next to a second controller and/or systemic oral corticosteroids (OCS) (Bel et al., 2011; Chung et al., 2014). This group represents 3.6%–10% of asthma patients and is known to have a high burden of disease with frequent asthma exacerbations and/or progressive lung function decline resulting in excessive utilization of health care resources (Barnes & Woolcock, 1998; Busse, Banks-Schlegel, & Wenzel, 2000; Hekking et al., 2015; O'Byrne, Naji, & Gauvreau, 2012).

The treatment approach of severe asthma is described in the Global Initiative for Asthma (GINA) guideline step 4 and 5. GINA step 4 ranges from medium to high dose ICS with a long acting beta-agonist (LABA), and/or an extra controller such as tiotropium, leukotriene modifier or theophylline. When asthma control is not achieved within GINA step 4, GINA step 5 advises add-on treatment including tiotropium, anti-IgE, anti-interleukin 5 (anti-IL5) and low dose OCS (“Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2016. Available from: www.ginasthma.org”). Driven by improved phenotyping, these add-on treatments are now increasingly prescribed

to distinct asthma phenotypes (Chung et al., 2014). For example anti-IgE can be considered in patients with a predominant allergic phenotype, and anti-IL5 in patients with a predominant eosinophilic phenotype (Trivedi, Pavord, & Castro, 2016). For patients with predominant chronic airflow obstruction or patients with unsatisfactory response to, or who are not eligible for anti-IgE or anti-IL5, Bronchial Thermoplasty (BT) can be considered (“British Thoracic Society, Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma, 2016. Available from: www.brit-thoracic.org.uk/document-library/clinical-information/asthma/btssign-asthma-guideline-2016”; “Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2016. Available from: www.ginasthma.org”; Trivedi et al., 2016).

Unfortunately, the mechanism of action of this relatively new, endoscopic treatment for asthma is incompletely understood. More insight in distinct phenotypes of asthma and pathways targeted by BT is needed to identify the asthma phenotype that benefits most of BT (Anderson, 2008; Gauthier, Ray, & Wenzel, 2015; Lotvall et al., 2011; Ray, Raundhal, Oriss, Ray, & Wenzel, 2016). Therefore, we review potential targets for BT, particularly those related to airway remodeling, including airway smooth muscle (ASM), extracellular matrix (ECM),

Abbreviations: ACQ, asthma control questionnaire; AHR, airway hyperresponsiveness; Anti-IL5, anti-interleukin 5; AQLQ, asthma quality of life questionnaire; ASM, airway smooth muscle; BAL, bronchoalveolar lavage; BT, Bronchial Thermoplasty; ECM, extracellular matrix; ED, emergency department; FEV₁, forced expiratory volume in 1 s; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroids; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-13, interleukin-13; IL-17, interleukin-17; LABA, long acting beta-agonist; NANC, non-adrenergic, non-cholinergic; OCS, oral corticosteroids; OCT, optical coherence tomography; PC₂₀, provocative concentration leading to a fall in FEV₁ of 20%; RANTES, Regulated on Activation Normal T Cell Expressed and Secreted; RBM, reticular basement membrane; RCT, randomized control trial; RF, radio-frequency energy; RML, right middle lobe; TGF-β, Transforming Growth Factor-Beta; TLD, targeted lung denervation; TNFα, tumour necrosis factor α

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inflammation, neural innervation and vascular function.

2. Airway remodeling and innervation in asthma

Asthma is characterized by airway remodeling which is defined as an alteration of the tissue structure and cells in the airways (James & Wenzel, 2007; Jeffery, 2001). Features of altered airway histology in asthma include a thickened epithelium, increased ECM including reticular basement membrane (RBM) thickening, ASM hyperplasia and hypertrophy, inflammation, vascular activation and angiogenesis and mucous gland hypertrophy (Bousquet et al., 1992; James & Wenzel, 2007; Jeffery, 2001).

2.1. Airway smooth muscle (ASM) in asthma

Key feature of airway remodeling in asthma is the altered ASM. This includes thickening of the ASM layer surrounding the airways, caused by ASM hypertrophy and hyperplasia as described in post-mortem specimens of patients with asthma (Bai, 1990; Carroll, Elliot, Morton, & James, 1993; Dunnill, Massarella, & Anderson, 1969; Hossain & Heard, 1970). More specific, James et al. found ASM hypertrophy in large airways in both non-fatal and fatal post-mortem asthma cases, whereas hyperplasia of ASM cells was present in the large and small airways in fatal asthma cases only (James et al., 2012).

Benayoun and colleagues assessed the degree of airway remodeling in bronchial biopsies from asthma patients ranging from intermittent to severe asthma in relation to controls and found that fibroblast accumulation and increase in ASM mass were associated with asthma severity (Benayoun, Druilhe, Dombret, Aubier, & Pretolani, 2003). Interestingly, it was shown that an increased ASM mass in pre-school wheezers can predict the development of asthma at school age (O'Reilly et al., 2013). In addition it has been reported that ASM in pre-schoolers with severe recurrent wheeze with atopy is increased as compared to pre-schoolers with severe recurrent wheeze without atopy. Nevertheless, no relation was found between inflammatory cell counts and the morphometrics of the airway wall (including vascularity, mucous gland area, RBM thickness and epithelial integrity) (Lezmi et al., 2015). These early findings of ASM alterations in pre-schoolers with severe recurrent wheeze with atopy, a group at high risk of developing asthma, make it plausible that airway remodeling including ASM alterations is an intrinsic feature of asthma and not solely a consequence of inflammation (Lezmi et al., 2015).

ASM contraction is considered the key effector of bronchoconstriction. Based on the increased sensitivity and increased response to bronchoconstrictive stimuli in asthma patients the ASM is likely to play an important role in airway hyperresponsiveness (AHR) (Dulin et al., 2003; Nair et al., 2017). However, the exact mechanism and role of the ASM in AHR is not completely understood. Most *in vitro* studies did not show an increase in maximal force-generating capacity of asthmatic tissues to various bronchoconstrictive stimuli (McParland, Macklem, & Pare, 2003). On the other hand, *in vitro* studies comparing ASM contractility of ASM cells and tissues from asthmatic and non-asthmatics, did find that the shortening of ASM of asthmatics is greater than that from non-asthmatics (Ma et al., 2002; McParland et al., 2003; Stephens, Li, Jiang, Unruh, & Ma, 2003). This suggests that the changed ASM and related increased contractility are more determined by the shortening ability instead of the maximal generating force capacity.

The principal function of ASM cells, contractile or synthetic, is likely to be determined by differentiation of ASM cells into different phenotypes as suggested by gene expression studies in patients with asthma and controls (Yick et al., 2013, 2014). Indeed, *in vitro* results demonstrated that exposure of ASM cells to a mitogenic stimulus results in a reversible modulation in phenotype from contractile to synthetic (Chamley-Campbell, Campbell, & Ross, 1979; Owens, 1995). Contractile phenotype ASM cells are characterized by high levels of contractile proteins and less biosynthetic organelles, and retain their ability

to contract. In contrast the ASM cells with a synthetic phenotype contain less contractile proteins and more biosynthetic organelles which may impair their ability to contract (Hirst, Walker, & Chilvers, 2000). This might be related to altered gene expression profiles of ASM observed in mild asthma patients with hyperresponsiveness (Yick et al., 2014). These physiological and biological findings in mild asthma patients require further investigation in patients with more severe asthma.

Next to the changes in amount, contractile and gene expression profiles of ASM observed in asthma described above, the ASM is considered an important source of inflammatory cytokines and chemokines (Chang, Bhavsar, Michaeloudes, Khorasani, & Chung, 2012; Hirst, 2003; Koziol-White & Panettieri, 2011). As such ASM might have a potential role in the activation and recruitment of various inflammatory cell types including neutrophils, eosinophils, T cells and mast cells (Hirst, 2003). Furthermore, pro-inflammatory cytokines and chemokines from ASM cells can induce AHR by enhancing ASM contraction and/or altering ASM relaxation (Black, Panettieri, Banerjee, & Berger, 2012) which may subsequently affect airway structural cells responsible for airway remodeling (Black et al., 2012; Koziol-White & Panettieri, 2011).

Another interesting feature of the ASM layer is that it has been described as a niche for mast cells (Brightling et al., 2002; Slats et al., 2007). It has been hypothesized that infiltration of these mast cells into the ASM layer is of importance as mast-cell products can cause 1) contraction and increase the responsiveness of ASM to bronchoconstrictive stimuli, 2) ASM proliferation and fibrosis and thereby contribute to airway remodeling and bronchoconstriction, 3) ASM pro-inflammatory cytokine release and surface protein expression (Page, Ammit, Black, & Armour, 2001). Brightling et al. demonstrated a significant difference between the number of mast cells in the ASM in patients with asthma and the number in both normal subjects and patients with eosinophilic bronchitis (Brightling et al., 2002). They also observed an inverse relation between the number of mast cells in the ASM of asthma patients and the provocative concentration of methacholine leading to a fall in forced expiratory volume in 1 s (FEV₁) of 20% (PC₂₀). In line with this observation, Slats et al. demonstrated an association between mast cell counts in the ASM layer and an impaired airway relaxation, thereby strengthening the potential contribution of mast cell – ASM interaction to AHR in asthma (Slats et al., 2007).

Altogether, the altered ASM in asthma patients is versatile and involved in multiple pathophysiological pathways of the asthmatic airway ranging from airway inflammation to airway mechanics. Although the true cause-effect between ASM, airway pathophysiology in asthma and functional/phenotypic features such as FEV₁ and AHR remains difficult to determine, ASM can certainly be seen as a key player.

2.2. Extra-cellular matrix (ECM) in asthma

Another component of airway remodeling is the ECM, which is increasingly envisioned as an important contributor to pathophysiological changes in airways diseases, including asthma. The ECM is a complex network of proteins, glycoproteins and lipids, produced by connective tissue cells including ASM, filling the extra-cellular space of the (sub)mucosa (Bousquet et al., 1992). The ECM contributes to the regulation of the airway and vascular diameter and prevent airways to collapse during expiration (Vignola, Kips, & Bousquet, 2000). However, next to this structural role, increased knowledge is gained about the role of the ECM on ASM, inflammatory cells and angiogenesis (Vignola et al., 2000). As such, in the airways of asthma patients, the profile of ECM proteins differs from those in the non-asthmatic airway. In the asthmatic airway it has been shown that there is enhanced deposition of collagens I, III and V, fibronectin, tenascin, hyaluronan, versican, laminin $\alpha 2/\beta 2$ and perlecan whereas decorin, collagen IV and elastin are decreased (Vignola et al., 2000). Also here, the ASM itself can contribute to the ECM by producing growth factors and ECM components

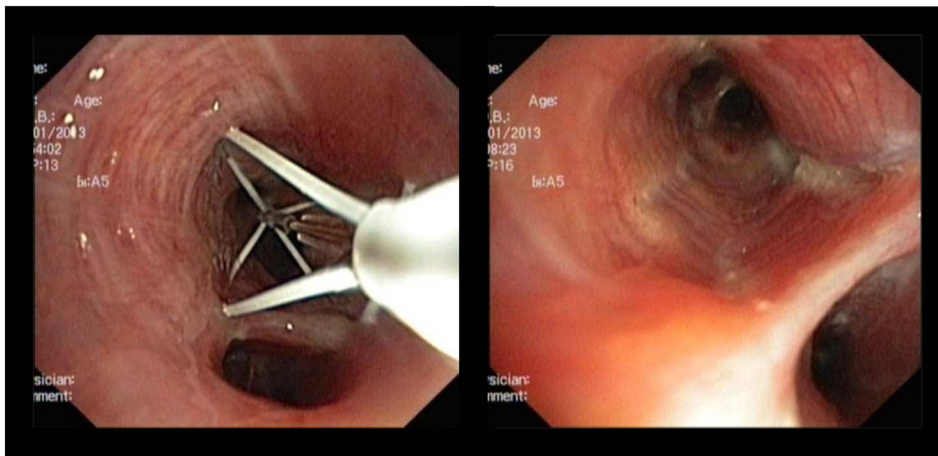


Fig. 1. Endoscopic view of asthmatic airways: left image shows the opened basket catheter during a BT activation resulting in RF energy delivery to a proximal airway; right image shows blanching of the mucosa of a proximal airway directly after BT.

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(Hirst, 2003; Vignola et al., 2000). Furthermore, the ECM deposited by asthmatic ASM *in vitro* enhances the proliferative capacity of the ASM cells (Johnson et al., 2004), which is in line with the observation that ASM hyperplasia is associated with an increase in ECM in asthma (James et al., 2012). Similarly, Arujo et al. reported increased fractions of elastin and fibronectin fibers in the ASM of asthma patients (Araujo et al., 2008). However, this is in contrast with observations of Yick et al. who could not demonstrate differences in ECM gene expression in biopsies of asthma patients and controls. Nevertheless, in asthma patients a relation between the fractional composition of the ECM in the ASM layer and the dynamics of airway function was detected (Yick et al., 2012), indicating that ECM components in the ASM layer contributes to the physiological phenotype of asthma. In summary, the ECM and ASM have important interactions which contribute to features of airway remodeling and function.

2.3. Airway innervation in asthma

The nerves also play an important role in bronchoconstriction and inflammation of the human airways (Groneberg, Quarcoo, Frossard, & Fischer, 2004; Pisi, Olivieri, & Chetta, 2009; Scott & Fryer, 2012). The human airway innervation includes a cholinergic, adrenergic and non-adrenergic, non-cholinergic (NANC) innervation system (Pisi et al., 2009). The cholinergic innervation consists of parasympathetic nerves that control primarily by signalling with acetylcholine upon muscarinic receptors which can be found on most inflammatory and airway structural cells including ASM and mucous glands. This parasympathetic signalling mediates bronchoconstriction, hypersecretion of mucus, inflammation and tissue remodeling (Kistemaker, Oenema, Meurs, & Gosens, 2012; Scott & Fryer, 2012). As such blocking the muscarinic receptors with inhaled anti-cholinergics, such as tiotropium, has been demonstrated to be clinically effective and indeed has been approved for treatment of poorly controlled asthma (Kerstjens et al., 2012). In COPD patients a new therapy ‘targeted lung denervation’ (TLD) that is based on ablation of parasympathetic pulmonary nerves surrounding the main bronchi is currently under investigation (Slebos et al., 2015). Theoretically this could be of benefit for asthma patients as well. The role of adrenergic innervation in regulating the bronchomotor tone is less known. After increasing airway resistance with propranolol in 21 asthma patients, blocking the α_2 -adrenergic receptor with tolazoline (α_2 -adrenergic receptor antagonist) did not cause changes in the bronchomotor tone in comparison to hexoprenaline (β_2 -adrenergic receptor agonist) and ipratropium (anticholinergic) (Islami et al., 2014), suggesting that this is not a primary mechanism in bronchomotor/bronchial tone (regulation). By mechanical, thermal, chemical or inflammatory stimuli, the NANC system releases neurotransmitters, including substance-P,

causing inflammation, mucus secretion, bronchoconstriction and vascular activation resulting in microvascular leakage (Boot et al., 2007; Groneberg et al., 2004). Indeed, inhaled substance P induces an increase in the severity of maximal airway narrowing to methacholine (Cheung, van der Veen, den Hartigh, Dijkman, & Sterk, 1994), which is indicative of a role of the NANC system in AHR. Summarizing, the different airway innervation systems, most importantly the cholinergic and NANC innervation systems, play a role in bronchomotor/bronchial tone regulation and inflammation of the airways.

3. Bronchial Thermoplasty

Bronchial Thermoplasty (BT) is an endoscopic, minimally invasive treatment for moderate-to-severe asthma based on local, radio-frequency (RF) energy delivery to the larger airways, developed to prevent excessive bronchoconstriction by reducing ASM (Cox, Miller, Mitzner, & Leff, 2004). As such BT is the first asthma treatment that targets airway remodeling instead of mainly modulating airway inflammation and bronchomotor tone. The treatment consists of 3 bronchoscopies during which consecutively the right lower lobe, the left lower lobe and the two upper lobes are treated. Through a standard bronchoscope a basket catheter is brought to airways with a diameter of ~2 mm–10 mm where it delivers RF energy generated by the Alair system (Boston Scientific, Natick, MA, USA) (Fig. 1). Each RF energy delivery or activation takes 10 s and heats the airway to 65°. Different sedation strategies are described that vary from mild midazolam sedation to general anaesthesia. Recently, sedation anaesthesiology nurse provided propofol-remifentanyl sedation has been described that combines high satisfaction and feasibility rates with a strong safety profile (d’Hooghe, Eberl, Annema, & Bonta, 2017).

Early animal studies showed a reduction in ASM mass at the area where BT was applied that correlated to an improved AHR with diminished contraction in response to methacholine (Danek et al., 2004). The first application of BT in humans was performed in patients with lung cancer who were scheduled to undergo lung resection. In this study it was shown that BT of the human, non-asthmatic airways was well tolerated and resulted in a significant alteration of ASM, an approximate 50% reduction, in treated airway (Miller et al., 2005).

4. Clinical efficacy of Bronchial Thermoplasty in asthma patients

The next step was to study BT in asthma patients. First, the safety and impact on lung function and AHR of BT over 2 years in 16 mild-to-moderate asthma patients was studied. Mild and moderate adverse events were observed within 1 week after the procedure which resolved spontaneously, with antibiotics or with a temporary increase of asthma medication. There were no severe adverse events. Additionally, an

improvement in AHR was found, that persisted until 2 years after BT treatment (Cox, Miller, McWilliams, Fitzgerald, & Lam, 2006). Next, in the AIR trial, an unblinded, randomized control trial (RCT), 112 moderate-to-severe asthma patients who had been treated with ICS/LABA were randomized to either BT treatment or a control group (Cox et al., 2007). Patients treated with BT showed a reduced rate of mild exacerbations as compared with baseline, which was unchanged in the control group. Furthermore there was a significant improvement in asthma control (asthma control questionnaire, ACQ) and quality of life (asthma quality of life questionnaire, AQLQ) in BT-treated patients as compared to controls. There was no significant difference in FEV₁ and the PC₂₀ methacholine. During the treatment period there was an increase in adverse respiratory events in patients treated with BT. Most frequently observed adverse events were dyspnea, wheezing, cough and chest discomfort. The majority of the adverse events occurred within 1 day after the procedure and resolved on average within 1 week. Severe adverse respiratory events were asthma exacerbation (n = 4), partial collapse of the left lower lobe (n = 1) and pleurisy (n = 1) which resulted in hospitalization (Cox et al., 2007).

The RISA trial, a randomized unblinded trial, included 34 severe asthma patients who had a significant improvement *versus* control subjects in quality of life (AQLQ), asthma control (ACQ) rescue medication use and pre-bronchodilator FEV₁% predicted. These results persisted when OCS and ICS were reduced, except for the pre-bronchodilator FEV₁% predicted. As a result 4 of 8 BT treated patients, compared to 1 of 7 control subjects were able to completely wean off OCS. Also this study showed a short-term increase in asthma related morbidity associated with BT (Pavord et al., 2007). In the largest, randomized double blind, sham-controlled AIR-2 trial, 297 moderate-to-severe asthma patients were randomized. 190 patients were randomized to the BT group and 98 were randomized to the sham control group, receiving 3 sham bronchoscopy procedures during which the BT treatment was mimicked. The primary endpoint demonstrated a clinically meaningful improvement in AQLQ score of 0.5 or greater in the BT treated patients, which, to a lesser extent was also found in the sham-controlled group, most probably due to placebo effect. However, a larger proportion of BT subjects compared with sham group subjects experienced a clinically meaningful within-subject improvement in AQLQ score of 0.5 or greater. Furthermore as secondary endpoints fewer severe exacerbations, emergency department (ED) visits and days missed from work were observed in the BT group. Likewise, an increase in respiratory adverse events was seen in the treatment period in patients treated with BT (Castro et al., 2010). Unfortunately, to date these studies were not able to define a specific asthma phenotype-based

responder profile. Furthermore, it should be noted that patients who have been included in the AIR and AIR2 trials were less severe than the patients included in the RISA trial and recent non-randomized trials (Castro et al., 2010; Chakir et al., 2015; Cox et al., 2007; Denner et al., 2015; Pavord et al., 2007; Pretolani et al., 2017).

4.1. Long-term results of Bronchial Thermoplasty

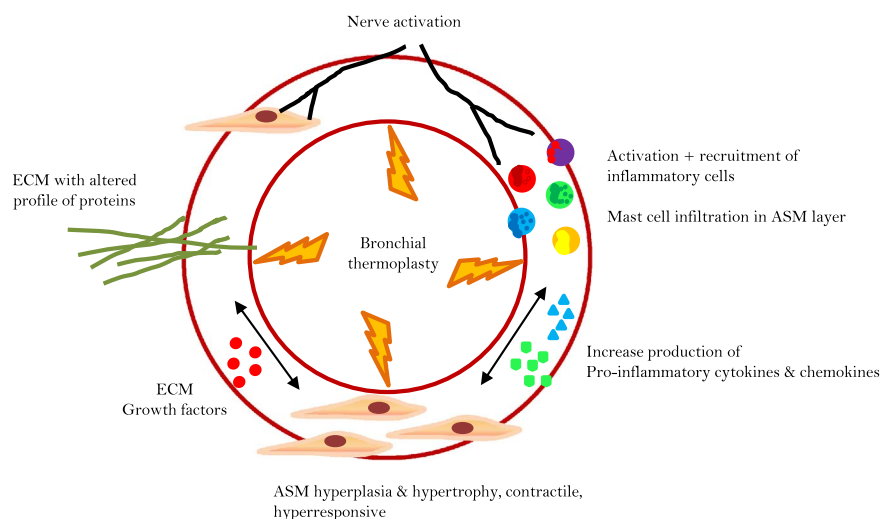
The 3 randomized trials conducted follow-up trials to address the efficacy and safety up to 5 years after BT. In the AIR long-term study, 43 BT treated patients were followed for 5 years and 24 control patients receiving standard of care were followed for 3 years. The rate of respiratory adverse events, hospitalization and ED visits remained stable over the 5 years following BT as compared to the year before BT. Similar results were found for the control group up to 3 years. Furthermore no deterioration in FEV₁ or radiological abnormalities were seen (Thomson et al., 2011).

The long-term results from the AIR-2 trial showed a sustained low proportion of subjects experiencing severe exacerbations and ED visits (average 5-year reduction in proportions: 44% for exacerbations and 78% for ED visits). Respiratory adverse events and hospitalizations related to asthma remained unchanged. FEV₁ remained stable and structural airway abnormalities were excluded by a HRCT up to 5 years after BT treatment (Wechsler et al., 2013).

In accordance with the AIR and the AIR-2 long-term studies, the follow-up study of the RISA trial patients demonstrated no deterioration in respiratory adverse events, FEV₁ or radiological abnormalities on chest X-ray. Furthermore, a persistent decrease in hospitalizations and ED visits was detected (Pavord et al., 2013). Unfortunately, the AIR-2 and the RISA trial did not have a control group to which the long-term result were compared. Taken together, the long term follow-up results of 3 randomized controlled trials are indicative that BT is safe, and reduces asthma exacerbations, ED visits and hospitalization for at least up to 5 years after BT treatment.

5. Emerging mechanisms underlying the clinical effectiveness of BT

Although demonstrated to be effective on clinical outcomes in randomized trials, the mechanism of the BT effect is incompletely understood. Several mechanisms could potentially underlie the clinical effectiveness of BT (Wilhelm & Chipps, 2016). More likely, a combination of different mechanisms will be responsible for the BT effect as the airways are a complex network of cells and structures with



above mentioned mechanisms.

Fig. 2. Schematic representation of airway pathophysiology in asthma and the related potential mechanisms of action of Bronchial Thermoplasty.

The different components of airway wall pathophysiology in asthma are illustrated. Airway smooth muscle (ASM) hyperplasia and hypertrophy (bottom) with increased contractility and hyperresponsiveness. Increased amounts of pro-inflammatory cytokines and chemokines produced by ASM can activate and recruit inflammatory cells. Pro-inflammatory cytokines and chemokines can contribute to airway hyperresponsiveness and impact airway structural cells. Another feature observed is mast cell infiltration in the ASM layer.

The ASM cells also produce extracellular matrix (ECM) growth factors and ECM proteins, which can affect the proliferative capacity of the ASM cells.

At the top of the figure, the airway innervation (cholinergic, adrenergic and non-adrenergic, non-cholinergic (NANC) systems) in asthma is illustrated. Nerves can contribute to bronchomotor/bronchial tone regulation and inflammation of the airways.

The lightning bolts in the lumen of the airway represent the four struts of the Bronchial Thermoplasty basket catheter that delivers RF energy to the airway wall with a potential effect on the

corresponding functions (Fig. 2).

5.1. Airway smooth muscle (ASM) reduction

Reducing the amount of airway smooth muscle, which is associated with asthma severity, may result in an improvement of asthma symptoms and control. Preclinical studies have shown that BT reduces ASM mass in canine model for asthma which was associated with a long term reduction of AHR. The effectiveness of BT on reducing the ASM mass in asthma patients was first demonstrated by Pretolani et al., analysing the ASM mass percentage (%) (ASM area as % of the total biopsy area) in airway biopsies 15 days before the first BT and 3 months after treatment with BT in 10 severe asthma patients. A reduction of ASM mass of 20.25% before BT to 7.28% (60%) after BT was found (Pretolani et al., 2014). Next, Denner et al. observed an ASM reduction of 38% at the first BT to 16% (58%) 6 weeks after the first BT (taken during the third BT) in 11 patients (Denner et al., 2015). This was confirmed with an ASM reduction of 12.9% at the first BT to 4.6% (64%) 3 weeks after the first BT (taken during the second BT) in another 17 patients (Chakir et al., 2015).

Most recently, Pretolani et al. confirmed reduction of ASM mass % (ASM area as % of the submucosal tissue area) from 19.7% to 5.2% (73%) in 15 severe asthma patients who were selected based on a baseline ASM mass of 15% or more (Pretolani et al., 2017). Limitation of the studies mentioned above is, that these were lacking an appropriate non-BT treated control group.

Despite this increasing evidence for the efficacy of BT on reducing the ASM, association with reductions in AHR are controversial. The earlier detected improvement of AHR in canines and the first 16 mild-to-moderate asthma patients treated with BT could not be reproduced in the AIR, RISA and AIR-2 trials (Castro et al., 2010; Cox et al., 2006, 2007; Pavord et al., 2007). Perhaps smaller dedicated studies with good standardized PC₂₀ measurements and well standardized BT administration rather than large multicentre RCTs would be able to show an association between ASM reduction and AHR. In addition, a large variation of asthma phenotypes and potentially associated ASM phenotypes (e.g. contractile and synthetic, distribution of ASM through the bronchial tree) included in the trials, could contribute to this lack of association. Emphasizing that a decreased amount of ASM cells does not automatically implies less contractility or decreased AHR, but that this may depend on the ASM cells phenotype/distribution regardless of the amount *per se*. Finally, PC₂₀ methacholine/histamine as a measure that is predominantly reflecting airway sensitivity to these agents may not be the appropriate outcome to assess the effects of BT on AHR. It is likely that the degree of maximal airway narrowing to inhaled methacholine or other stimuli is a much better outcome of any changes in ASM contractility (Sterk & Bel, 1989). However, changes in maximal airway narrowing can only be quantified safely in patients with mild asthma (Booms, Cheung, Timmers, Zwinderman, & Sterk, 1997).

Next to a reduction of ASM itself, any loss of contractile and relaxation function of ASM could play a role. In bovine ASM exposure to extreme temperatures (> 55 °C) led to complete inhibition of the contractile ASM function. This phenomenon occurred prior to necrosis or apoptosis and appears to involve a disruption of the myosin. The relaxation function was unchanged by thermal exposure (Dyrda et al., 2011). It is beyond doubt that BT affects the structure of airways and the surrounding tissue after exposure to high temperature, as is confirmed by early radiological abnormalities reaching far peripheral to the treated airway, seen on low dose CTs obtained 24 h after BT (Debray et al., 2017; d'Hooghe, van den Berk, Annema, & Bonta, 2017).

Overall, a reduction in ASM following BT is plausible and demonstrated in multiple studies. However, how the reduction of ASM relates to the observed clinical effects after BT is largely unknown. Whether ASM mass can be used as a predictive marker in selecting patients that benefit from BT treatment needs to be determined.

5.2. Airway epithelium

The described extensive radiological abnormalities seen directly after BT reinforce that BT certainly modifies the airways (Debray et al., 2017; d'Hooghe, van den Berk, et al., 2017). During BT, the airway epithelium is the first structure that the RF energy is delivered to which can be noticed by blanching of the mucosa. The effect of BT on the epithelium was investigated by Pretolani et al. The amount of epithelial neuroendocrine PGP-positive cells in the epithelial layer was diminished after BT (Pretolani et al., 2017). No impact of BT was found on the proportion of regenerating epithelium or normal stratified columnar, metaplastic, or squamous epithelium. More data are needed to look into the modifying effect of BT on the airway epithelium.

5.3. Inflammation

Since ASM produces pro-inflammatory cytokines and chemokines itself, any BT induced reduction of ASM would imply a reduction of those compounds in the airway wall. This potential effect of BT on airway inflammation is another mechanism that could contribute to the benefits of BT. One study investigated cytokines associated with asthma airway inflammation in bronchoalveolar lavage (BAL) 3 and 6 weeks after BT treatment and found a significant reduction of TGF- β and RANTES whereas other asthma key cytokines including eotaxin, IL-4, IL-5, IL-6, IL-13 and IL-17 were unchanged (Denner et al., 2015). Also the percentage of eosinophils appeared to be significantly reduced. The possible influence of the (understandable) prophylactic high dose of prednisolone given shortly before the sampling is however a potential bias of this study (Denner et al., 2015). In airway biopsies taken 3 months after BT, Pretolani et al. found no effect of BT on mucosal eosinophils and neutrophils (Pretolani et al., 2017).

Furthermore, the observed increase of mast cells within the ASM layer in asthma and its relationship with increased AHR (Brightling et al., 2002) and impaired airway relaxation (Slats et al., 2007) implies that reducing the mast cells by ablation of ASM niche of these cells could play a role in the mechanism of action of BT. To the best of our knowledge there are no data on mast cell numbers within ASM before and after BT. Considering the pivotal role of the mast cell in atopic asthma, one might hypothesize that atopic asthma patients could be high responders for BT. In the currently ongoing TASMA study (ClinTrials.gov nr: NCT02225392) the effect of BT on ASM and related airway inflammation will be further investigated.

Is there any effect of BT on systemic inflammation? A retrospective review in 10 BT treated severe asthma patients showed a significant reduction in blood eosinophil count 1 year after BT treatment (Ryan, Fowler, & Niven, 2016). The question whether a BT-induced effect can be expected on airway inflammation and which inflammatory cells are modulated largely remains unanswered.

5.4. ECM

Is there any role for ECM in the effects of BT in asthma? In 17 severe asthma patients a decreased type 1 collagen deposition was described shortly after the first BT when examined at subsequent BT treatments (Chakir et al., 2015). This effect remained unchanged after 27 months in 9 of these BT treated patients who consented for another bronchoscopy (Salem et al., 2016). A decrease in thickness of the subepithelial reticular basement membrane (RBM) was confirmed by Pretolani et al., however these authors also observed a significantly larger proportion of the biopsy area stained for collagen (Pretolani et al., 2017). Considering these partly opposing results regarding the effect of BT on ECM larger sample sizes with non-BT treated controls are needed. Furthermore, the mechanism how BT impacts on ECM is unknown. It could be postulated that BT directly effects the ECM composition or that BT indirectly affects ECM composition by reducing the ECM-protein-producing ASM and other structural cells. Considering the important interaction

between the ECM and ASM described above, BT induced changes in the ECM may contribute to the efficacy of BT.

5.5. Neural innervation and vascular function

What is the evidence of BT impact on neural or vascular structure? Impact of BT on the neural innervation was found in airway biopsies 3 months after BT by a single study by Pretolani et al. Both submucosal and ASM associated nerve fibers were significantly reduced and a 95% reduction of neuroendocrine epithelial cells was detected. Interestingly, also the non-BT treated right middle lobe (RML) showed a down-regulation of these neuroendocrine epithelial cells. No significant effect was seen on the density of submucosal blood vessels (Pretolani et al., 2017). More studies are needed to elucidate the BT induced effects on neural innervation and vascularisation. It might even be postulated that any BT effects on neural innervation or vascularisation is spreading out to distal untreated airway areas, even though BT is delivered to the larger airways (airway diameter ~2 mm and larger). Considering the anatomy of the neural innervation and vascularisation system running along the entire bronchial tree from proximal to the smallest distal airways, it is imaginable that a BT-induced proximal interruption in the larger airways impacts the smaller distal airways.

6. Future perspectives

The available data on the potential mechanisms underlying the clinical effectiveness of BT makes it unlikely that only one feature of airway pathophysiology in severe asthma is modulated by BT for the observed clinical benefits. The effectiveness of BT is more likely a consequence of its impact on a complex interplay between various airway pathophysiological components of which airway remodeling including ASM is a key player. Results are awaited from large registries and (randomized) trials, such as the TASMA trial (Clin.Trials.gov nr: NCT02225392), investigating the mechanism of action of BT and trying to define clinical parameters and biomarkers that differentiate the responders from the non-responders of this therapy. The latter would be very valuable for selecting patients to receive this novel treatment modality in this heterogeneous disease. Extended severe asthma phenotyping might therefore contribute to further personalized asthma treatment by BT. Next to phenotyping by clinical, molecular, pathophysiological and functional parameters as described above, also novel high resolution imaging techniques, such as optical coherence tomography (OCT) (Huang et al., 1991) might contribute to this. Indeed OCT has been shown effective in *in-vivo* quantification of airway wall dimensions, remodeling and even ASM content (Adams et al., 2016; Chen et al., 2015; Coxson & Lam, 2009; Coxson et al., 2008; Ding et al., 2016). Therefore, OCT can potentially contribute to severe asthma phenotyping with a focus on airway remodeling and ASM mass and serve as a possible instrument for patient selection for BT and/or monitoring treatment response after BT (Kirby et al., 2015). In addition there could even be a role of BT-induced changes in bronchial microbiome as potential modulator of the clinical control of asthma (Sullivan, Hunt, MacSharry, & Murphy, 2016).

7. Conclusions

Bronchial Thermoplasty is an endoscopic treatment for moderate-to-severe asthma patients who are uncontrolled despite optimal medical treatment. By local RF energy delivery to the airways BT aims to reduce the ASM mass, one of the key features of airway remodeling. However, the mechanism of action of BT is likely to be more complex than solely the ASM reduction and is still incompletely understood. More results are needed to elucidate the effect of BT on airway pathophysiology including airway remodeling and innervation, which will contribute to define the severe asthma phenotype that benefits most of this novel treatment modality..

Conflict of interest statement

Dr. P.I. Bonta has received honoraria from Boston Scientific for lectures about Bronchial Thermoplasty.

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